DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD
DASH.HARVARD.EDU

HARVARD LIBRARY
Office for Scholarly Communication

Notch signaling mediates p63-induced quiescence: A new facet of p63/Notch crosstalk

Citation
Forster, Nicole, and Leif W. Ellisen. 2011. "Notch signaling mediates p63-induced quiescence: A new facet of p63/Notch crosstalk." Cell Cycle 10 (21): 3632-3633. doi:10.4161/cc.10.21.18182. http://dx.doi.org/10.4161/cc.10.21.18182.

Published Version
doi:10.4161/cc.10.21.18182

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:11717641

Terms of Use
This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. Submit a story .

Accessibility

Cell Cycle 10:21, 3627-3635; November 1, 2011; © 2011 Landes Bioscience

Cell Cycle News & Views

Do neuroendocrine cells come up large in small cell lung cancer?

Comment on: Park KS, et al. Cell Cycle 2011; 10:2806–15

Andrew S. Goldstein and Jiaoti Huang; University of California, Los Angeles; Los Angeles, CA USA;
Email: AGoldstein@mednet.ucla.edu and JiaotiHuang@mednet.ucla.edu; http://dx.doi.org/10.4161/cc.10.21.17688

Most organs of the body can develop tumors of different histological subtypes that often show distinct biologic behavior and respond differently to various therapeutic modalities. Understanding the cellular and molecular components driving each subtype will make it possible to develop specific targeted therapies tailored to each tumor type to maximize therapeutic efficacy and minimize side effects. One critical issue is whether distinct tumor subtypes arising in the same organ originate from distinct cell types or from a common cell of origin. Two recent studies, including the one from Carla Kim, Julien Sage and colleagues,[1] use mouse models to demonstrate that small cell lung cancer (SCLC) does not arise from the same cell of origin as non-small cell lung cancer (NSCLC). While NSCLC can arise from transformed Clara cell-specific protein (CCSP)-expressing cells,[2] SCLC most likely arises from CCSP-negative neuroendocrine (NE) cells.

SCLC is an aggressive, less common histological subtype of lung cancer composed of highly proliferative tumor cells showing morphological features of NE cells and expressing NE markers, including synaptophysin, chromogranin and Calcitonin gene-related protein (CGRP). SCLC is associated with mutations in tumor suppressor genes *Rb* and *p53* and is histologically and molecularly distinct from the more common NSCLC, which is often characterized by mutations in K-ras and expression of the alveolar type II (AT-2) marker surfactant protein C (SP-C).[4]

Park et al. and Sutherland et al. demonstrated that deletion of *Rb* and *p53* in all cells led to proliferating NE-like lesions and SCLC.[1,4] When *Rb* and *p53* were deleted specifically in CCSP-expressing cells, excluding NE cells and predominantly targeting the Clara cell lineage, SCLC was not observed.[1,4] Since CCSP-expressing cells can initiate NSCLC but not SCLC, the lung must contain multiple cell populations that are susceptible to transformation.

The main inconsistency between the two studies is presented when *Rb* and *p53* were deleted in SP-C+ cells, predominantly representing the alveolar lineage. Kim and colleagues observed the development of rare adenocarcinoma, but not SCLC, when using both adenoviral delivery and a double-transgenic approach to delete *Rb* and *p53* in alveolar cells.[1] In contrast, Sutherland et al. reported that 50% of animals developed SCLC after loss of *Rb* and *p53* in cells expressing SP-C, but the tumors took longer to develop than for those initiated in CGRP+ NE cells.[4]

Sutherland et al. showed that CGRP+ NE cells were the most efficient cells of origin for SCLC, although other cell types could also contribute to disease initiation.[4] Park et al. exclude non-NE cell types (CCSP+ and SP-C+ cells) in the initiation of SCLC but do not definitively demonstrate a NE cell origin,[1] leaving open the possibility of a yet-to-be-discovered cell of origin. This emerging concept that tumors of different histological types arising in the same organ may originate from different adult tissue cell types is also supported by recent evidence in breast cancer.[5]

After lung cancer, the most common cancer site for males is the prostate, with the acinar type adenocarcinoma being the predominant histologic type. Prostatic small cell neuroendocrine carcinoma (SCNC) is a rare histological variant that is distinguished by proliferating NE-like cells.[6] SCNC is aggressive and does not respond to hormonal therapy in contrast to adenocarcinoma comprised of luminal type tumor cells. Deletion of the same tumor suppressor pathways (*Rb* and *p53*) in the prostate[7] leads to SCNC-like lesions. We recently showed that target cells expressing basal cell markers, which are generally absent from prostate adenocarcinoma, are efficient cells of origin for luminal type prostate adenocarcinoma.[8] Basal stem/progenitor cells in the prostate have the capacity to differentiate into NE cells,[9] suggesting that they may also be capable of SCNC initiation. Another possibility is that NE cells, a component of normal human prostate and adenocarcinoma, can initiate SCNC in the prostate, similar to results by Berns and colleagues.[3]

These important studies demonstrate that different histological subtypes of lung cancer can arise from distinct cell types. This principle is likely to hold true in tumors arising in other organs. Further interrogation of different target cells and the mechanisms that they utilize during progression to distinct cancer types may inform the development of future biomarkers to monitor the disease and potentially lead to new therapeutic targets.

©2011 Landes Bioscience
Do not distribute

References

1. Park, KS et al. Cell Cycle 2011; 10:2806-15; PMID:21822053; http://dx.doi.org/10.4161/cc.10.16.17012.
2. Ji H, et al. Oncogene 2011; 200:2105-12.
3. Meuwissen R, et al. Cancer Cell 2003; 4:181-9; PMID:14522252; http://dx.doi.org/10.1016/S1535-6108(03)00220-4.
4. Sutherland KD, et al. Cancer Cell 2011; 19:754-64; PMID:21665149; http://dx.doi.org/10.1016/j.ccr.2011.04.019.
5. Lim E, et al. Nat Med 2009; 15:907-13; PMID:19648928; http://dx.doi.org/10.1038/nm.2000.
6. Huang J, et al. Anal Quant Cytol Histol 2007; 29:128-38; PMID:17672372.
7. Zhou Z, et al. Cancer Res 2006; 66:7889-98; PMID:16912162; http://dx.doi.org/10.1158/0008-5472.CAN-06-0486.
8. Goldstein AS, et al. Science 2010; 329:568-71; PMID:20671189; http://dx.doi.org/10.1126/science.1189992.
9. Goldstein AS, et al. Proc Natl Acad Sci USA 2007; 105:20882-7; http://dx.doi.org/10.1073/pnas.0811411106.

SUMO bridges Elg1 and SUMO interactors

Comment on: Parnas O, et al. Cell Cycle 2011; 10:2894–903

Lisa E. Hang and Xiaolan Zhao*; Memorial Sloan-Kettering Cancer Center and Cornell University; New York, NY USA;
*Email: zhaox1@mskcc.org; http://dx.doi.org/10.4161/cc.10.21.18032

SUMO is a small protein that can be conjugated to other proteins and subsequently modulates their functions. The effects of SUMO on target proteins can be diverse and dynamic, making it a useful means for altering protein functions and regulating cellular pathways. SUMO plays an important role in genome maintenance. Multiple studies have shown that mutating sumoylation or desumoylation enzymes can lead to sensitivity to DNA damaging agents as well as defects in double-strand DNA break repair and in the maintenance of specific genomic regions.[1] The underlying mechanisms accounting for these defects have begun to emerge from studies on how SUMO modification alters the functions of DNA metabolism enzymes and how proteins that can noncovalently bind to SUMO influence repair processes.

Elg1 is the large subunit of one of the three alternative replication factor C (RFC) complexes.[2] Because the canonical RFC loads PCNA, which facilitates DNA polymerases and docks other DNA processing enzymes on DNA, Elg1-RFC is also thought to influence PCNA functions. Interestingly, the budding yeast Elg1 contains three SUMO interacting motifs (SIMs) at its N-terminal region and noncovalently binds to SUMO.[3] Recent work has suggested that the yeast Elg1 preferentially binds to sumoylated PCNA and may help to unload this form of PCNA from chromatin.[3] As sumoylated PCNA can interact with the anti-recombinase Srs2, the potential removal of SUMO-PCNA by Elg1 could bias towards the use of recombinational repair over other types of repair, particularly those mediated by PCNA ubiquitination.

In the September 1st issue of *Cell Cycle*, Parnas and colleagues described the identification of seven additional Elg1-interacting proteins using a yeast two-hybrid screen.[4] Like Elg1, the seven proteins all contain SIMs and are previously identified SUMO interactors.[5] This finding raises the possibility that the observed Elg1 interactions with SIM-containing proteins are bridged by poly-sumoylated proteins. In support of this idea, the authors showed that fragments containing the SIMs of the seven Elg1-interacting proteins can associate with Elg1, and at least one of these interactions requires poly-sumoylation. Although the bridging protein(s) are yet to be identified, the authors show that it is not PCNA.

Bridging protein-protein interactions is a reccurring theme for SUMO's effects. For example, SUMO was found to mediate multilateral protein interactions required for PML body formation.[6] This work by Parnas et al. suggests that Elg1 may exert some of its effects via interactions with other SIM-containing proteins. However, since mutants of most of the identified Elg1 interactors do not exhibit a phenotype similar to *elg1Δ*, they may not be the physiological Elg1 partners.

One potential exception is the Slx5/8 complex, which also affects genomic stability. The best-characterized biochemical activity of the Slx5/8 complex is as a ubiquitin ligase function with a higher affinity for sumoylated proteins.[7,8] A phenotype often indicative of mutants defective in this type of ubiquitin ligase function is the accumulation of poly-sumoylated proteins. However, unlike *slx5Δ* or *slx8Δ*, *elg1Δ* cells do not exhibit this defect. Conversely, unlike *elg1Δ*, *slx5Δ* cells contain normal levels of sumoylated PCNA. These results as well as other tests, including recombination and mutation assays, suggest that Elg1 and the Slx5/8 complex have non-overlapping functions. Therefore, the physiological role of the SUMO-mediated Elg1-Slx5/8 interaction remains to be determined. Genetic studies using alleles that specifically impair this interaction will be informative. In addition, seeking the sumoylated bridging proteins that mediate the interaction between Elg1 and Slx5/8 will also provide important clues about their roles in genome maintenance. Nevertheless, this work suggests that some aspects of Elg1's function may rely on its interaction with other SUMO interactors. Since the lack of human Elg1 leads to a set of defects similar to those seen in yeast *elg1Δ* cells,[9] it will be interesting to consider the greater impact of the potential ability of Elg1 to interact with other SUMO interactors.

©2011 Landes Bioscience
Do not distribute

References

1. Bergink S, et al. Nature 2009; 458:461-7; PMID:19325626; http://dx.doi.org/10.1038/nature07963.
2. Aroya SB, et al. DNA Repair (Amst) 2005; 4:409-17; PMID:15725622; http://dx.doi.org/10.1016/j.dnarep.2004.08.003.
3. Parnas O, et al. EMBO J 2010; 29:2611-22; PMID:20571511; http://dx.doi.org/10.1038/emboj.2010.128.
4. Parnas O, et al. Cell Cycle 2011; 10: 2894-2903; PMID:21869594; http://dx.doi.org/10.4161/cc.10.17.16778.
5. Hannich JT, et al. J Biol Chem 2005; 280:4102-10; PMID:15590687; http://dx.doi.org/10.1074/jbc.M413209200.
6. Shen TH, et al. Mol Cell 2006; 24:331-9; PMID:17081985; http://dx.doi.org/10.1016/j.molcel.2006.09.013.
7. Mullen JR, et al. J Biol Chem 2008; 283:19912-21; PMID:18499666; http://dx.doi.org/10.1074/jbc.M802690200.
8. Xie Y, et al. J Biol Chem 2007; 282:34176-84; PMID:17848550; http://dx.doi.org/10.1074/jbc.M706025200.
9. Lee KY, et al. J Biol Chem 2010; 285:10362-9; PMID:20147293; http://dx.doi.org/10.1074/jbc.M109.092544.

Caveolin-1: A new therapeutic target in tissue fibrosis and scleroderma?

Comment on: Castello-Cros R, et al. Cell Cycle 2011; 10:2140–50

Francesco Del Galdo; University of Leeds; Leeds, UK; Email: f.delgaldo@leeds.ac.uk; http://dx.doi.org/10.4161/cc.10.21.18033

Scleroderma or systemic sclerosis (SSc) is the most common systemic fibrotic disease. Its puzzling pathogenesis, including tissue fibrosis, fibroproliferative vasculopathy and autoimmune activation, has yet to be unraveled and is still an open challenge for the scientific community.[1] As a consequence, the clinical management of patients affected by this condition is still devoid of effective interventions able to block the progression of the tissue fibrosis.[2]

In the last decade, several factors and signaling pathways have been implicated in the pathogenesis of tissue fibrosis.[3] Conflicting studies from different group may reflect (1) the lack of a comprehensive experimental model for scleroderma and/or (2) redundancy in the mechanism(s) that lead to tissue fibrosis.

The latter is a more likely explanation in light of recent studies linking caveolin-1 down-regulation with tissue fibrosis in scleroderma as well as other fibrotic conditions.[4,5] Caveolin-1 (Cav-1) is the major coat protein of caveolar lipid rafts. These flask-shaped plasma membrane invaginations function as microdomains that are able to integrate and regulate the signaling of receptor-ligand complexes, including TGF-β,[5] PDGF, VEGF[6] and Endothelin.[7] These pathways have all been shown to promote the pathogenesis of SSc.[3] Moreover, mice lacking caveolin-1 develop skin and lung tissue fibrosis and pulmonary artery hypertension, all the major clinical hallmarks associated with scleroderma.[4,5] A recent article by Castello-Cros et al. provides further evidence for the use of Cav-1-knockout mice as an accurate pre-clinical model for scleroderma. The authors show that tissue fibrosis observed in Cav-1-null mice is accompanied by decreased collagen fiber diameter and increased tensile strength in the skin, both well-known characteristics of scleroderma. Indeed, the observation that a single gene defect causes such a dramatic structural change in overall skin architecture highlights the importance of caveolin-1 in regulating extracellular matrix homeostasis. Most importantly, the study by Castello-Cros et al. also opens two new venues of investigation in SSc that were not previously considered.

The first is that lack of caveolin-1 expression may be causing the subtle chronic tissue inflammation observed in SSc. Previous hypotheses regarding the pathogenesis of scleroderma have focused on autoimmune activation as the primary event in scleroderma, which, in turn, causes profibrotic activation and vasculopathy.[1]

©2011 Landes Bioscience. Do not distribute

Caveolin-knockout mice do not display autoantibodies nor other signs of autoimmunity. However, in the manuscript by Castello-Cros et al., the authors observed an increased number of tissue macrophages and mast cells in the skin. In this regard, it is interesting to speculate that fibroblastic changes in scleroderma (e.g., decreased caveolin-1 expression) may drive the autoimmune reaction rather than being its consequence.

A second important point is the observation of autophagic and mitophagic features in tissue fibroblasts from Cav-1-knockout mice. Recent studies have stressed the importance of autophagy in the "lethal" cancer microenvironment.[8] Given the known similarities between cancer-associated fibroblasts and profibrotic fibroblasts,[9] it is likely that cancer-associated fibroblasts and Cav-1-negative fibroblasts in scleroderma patients display a similar metabolic signature. The exact role of this metabolic shift in promoting the profibrotic phenotype has yet to be determined, but these new findings may lead to innovative therapeutic approaches for targeting tissue fibrosis.

References

1. Jimenez SA, et al. Ann Intern Med 2004; 140:37-50; PMID:14706971.
2. Kowal-Bielecka O, et al. Ann Rheum Dis 2009; 68:620-8; PMID:19147617; http://dx.doi.org/10.1136/ard.2008.096677.
3. Gabrielli A, et al. N Engl J Med 2009; 360:1989-2003; PMID:19420368; http://dx.doi.org/10.1056/NEJMra0806188.
4. Del Galdo F, et al. Curr Opin Rheumatol 2008; 20:713-9; PMID:18949888; http://dx.doi.org/10.1097/BOR.0b013e3283103d27.
5. Del Galdo F, et al. Arthritis Rheum 2008; 58:2854-65; PMID:18759267; http://dx.doi.org/10.1002/art.23791.
6. Tahir SA, et al. Cancer Biol Ther 2009; 2009:2286-96.
7. Hassan GS, et al. Am J Physiol Heart Circ Physiol 2006; 290:H2393-401; PMID:16415072; http://dx.doi.org/10.1152/ajpheart.01161.2005.
8. Witkiewicz AK, et al. Cell Cycle 2011; 10:1794-809; PMID:21521946; http://dx.doi.org/10.4161/cc.10.11.15675.
9. Sotgia F, et al. Am J Pathol 2009; 174:746-61; PMID:19234134; http://dx.doi.org/10.2353/ajpath.2009.080658.

Neuroendocrine cells: Potential cells of origin for small cell lung carcinoma

Comment on: Park KS, et al. Cell Cycle 2011; 10:2806–15

Chieh-Yang Cheng and Alexander Y. Nikitin*; Cornell University; Ithaca, NY USA; *Email: an58@cornell.edu; http://dx.doi.org/10.4161/cc.10.21.18034

Small cell lung carcinoma (SCLC) is the most aggressive type of lung cancer, accounting for at least 14% of new lung cancer cases.[1] SCLC is characterized by expression of neuroendocrine markers, such as synaptophysin and calcitonin gene-related peptide. However, about 10% of SCLCs have areas of other lung cancer types, such as squamous cell carcinoma and adenocarcinoma. This observation suggests that, in addition to neuroendocrine cells, SCLC may originate from some not-yet-identified, multi-/bipotent stem/progenitor cells, which are able to differentiate into several cell types, including neuroendocrine cells. Alternatively, SCLC may be a result of phenotypical plasticity of other pulmonary non-neuroendocrine cells, such as Clara cells, which can generate ciliated cells of the lower airways, alveolar type 2 (AT2) cells, which are able to differentiate toward alveolar type 1 (AT1) cells of the alveolar ducts and acini or bronchio-alveolar stem cells (BASCs), which can give rise to Clara, AT1 and AT2 cells (**Fig. 1**).

In their study, groups led by Julien Sage and Carla Kim[2] addressed the issue of the cell of SCLC origin by taking advantage of previous observations that Cre-*loxP*-mediated

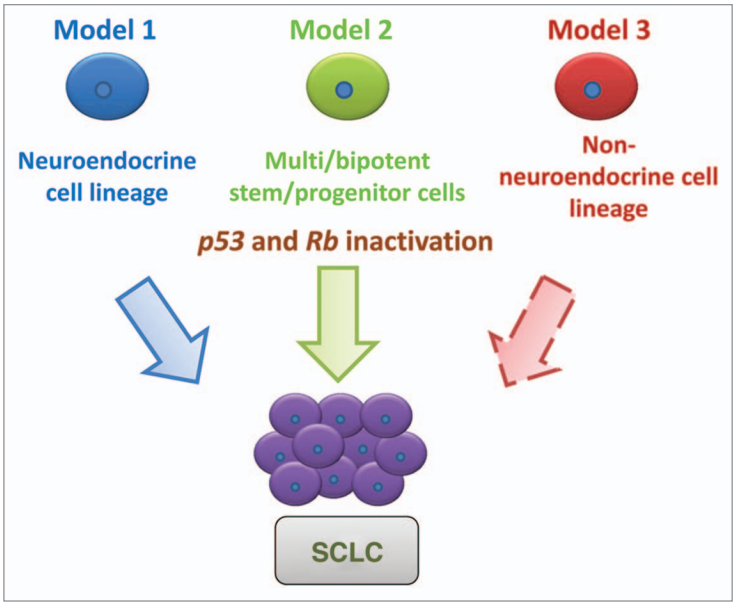


Figure 1. Models of potential origin of SCLC. Model 1 proposes that unipotent stem/progenitor cells or mature cells of neuroendocrine cell lineage give rise to SCLC. This model anticipates that cells lacking p53 and Rb may differentiate toward other types of lung cancer, such as adenocarcinoma and squamous cell carcinoma in some cases. Model 2 proposes that SCLC arises from stem/progenitor cells able to differentiate toward several cell types, including neuroendocrine cells. Model 3 suggests that at least some of SCLCs could arise from non-neuroendocrine cell lineages due to differentiation plasticity of cells deficient for p53 and Rb.

©2011 Landes Bioscience
Do not distribute

inactivation of the tumor suppressor genes *p53* and *Rb* in mouse lungs resulted in neuroendocrine malignancies closely resembling human SCLC according to their morphological and molecular properties.[3] Since *p53* and *Rb* alterations are among the most common mutations in human SCLC,[1] this mouse model is likely to accurately reflect many facets of the disease pathogenesis.

By expressing Cre recombinase in different cell types of the lung, the authors determined that neither Clara cells nor AT2 cells nor BASCs gave rise to neuroendocrine neoplasms. The authors concluded that neuroendocrine cells were the most likely cells of origin of SCLC. A similar conclusion was recently reached by the group of Anton Berns.[4] However, at variance with the study by Park et al.,[2] Sutherland et al.[4] observed that deletion of *p53* and *Rb* in AT2 cells does result in neuroendocrine neoplasms, albeit at lower rate (45 vs. 83%) and after increased median latency (452 vs. 362 d), as compared with neuroendocrine cell-specific deletion of these genes. As Park et al. note, the difference in observations can be explained by different efficiency of adenoviruses used by the two groups. However, it is less clear why mice expressing Cre under the control of AT2 and BASC-specific surfactant protein C (SPC) promoter have not developed neuroendocrine neoplasms similar to those initiated by SPC-Cre adenovirus used by Sutherland et al. and Park et al. propose that constitutive deletion of *p53* and *Rb* in transgenic mice may affect lung cells differently than acute deletion of these genes after administration of SPC-Cre adenovirus. It is also possible that unlike the 4.8 kb fragment of mouse SPC promoter used in the adenoviral vector, the 3.7 kb human SPC promoter fragment used in transgenic mice does not drive Cre expression in cells capable of neuroendocrine differentiation. Another possibility is that the high levels of adenoviral infection may affect the degree of cell susceptibility to increased differentiation plasticity after *p53* and *Rb* inactivation, either directly or by initiating microenvironmental changes. Finally, a difference in genetic backgrounds of mice used by different groups could be a possible reason. Future studies based on additional, preferably not adenovirus-based, systems should clarify this discrepancy.

Taken together, the studies by Park et al. and Sutherland et al. make an important step toward better understanding of SCLC pathogenesis by strengthening the possibility that neuroendocrine cell lineage is the primary source of SCLC while convincingly excluding Clara cells as a potential cell of origin. At the same time, these studies also highlight the need for better tools allowing characterization of pulmonary neuroendocrine cell lineage during ontogenesis. A recent report has indicated the existence of mouse embryonic stem/progenitor cells, which are able to differentiate toward all epithelial cell types, including neuroendocrine cells.[5] Unfortunately, there has been no direct evidence that neuroendocrine cells are among the progeny of recently identified putative mouse adult lung stem/progenitor cells.[6,7] It is likely that further progress in these areas will firmly establish the cell of origin for SCLC, thereby facilitating development of new rationally designed diagnostics and therapeutics.

References

1. Jackman DM, et al. Lancet 2005; 366:1385-96; PMID:16226617; http://dx.doi.org/10.1016/S0140-6736(05)67569-1.
2. Park K-S, et al. Cell Cycle 2011; 10:2806-15; PMID:21822053; http://dx.doi.org/10.4161/cc.10.16.17012.
3. Meuwissen R, et al. Cancer Cell 2003; 4:181-9; PMID:14522252; http://dx.doi.org/10.1016/S1535-6108(03)00220-4.
4. Sutherland KD, et al. Cancer Cell 2011; 19:754-64; PMID:21665149; http://dx.doi.org/10.1016/j.ccr.2011.04.019.
5. Rawlins EL, et al. Development 2009; 136:3741-5; PMID:19855016; http://dx.doi.org/10.1242/dev.037317.
6. Kim CF, et al. Cell 2005; 121:823-35; PMID:15960971; http://dx.doi.org/10.1016/j.cell.2005.03.032.
7. McQualter JL, et al. Proc Natl Acad Sci USA 2010; 107:1414-9; PMID:20080639; http://dx.doi.org/10.1073/pnas.0909207107.

The importance of mitochondrial fusion in aging

Comment on: Scheckhuber CQ, et al. Cell Cycle 2011; 10:3105–10

Cristina Mazzoni* and Claudio Falcone; Sapienza Università di Roma; Rome, Italy; *Email: cristina.mazzoni@uniroma1.it; http://dx.doi.org/10.4161/cc.10.21.18181

Increasing evidences indicates that mitochondria, besides their role in supplying cells with ATP through oxidative phosphorylation, play a relevant role in the regulation of apoptosis and cellular aging from mammals to yeast. Mitochondria are highly dynamic organelles whose number, morphology and distribution change in response to cellular activity, nutritional status and developmental programs. Mitochondrial dynamicity involves two opposite events, namely fission and fusion, and intrinsic or extrinsic disturbance of such processes might results in cellular physiological alterations and diseases.

As an example, the autosomal dominant optic atrophy and the neurodegenerative Charcot-Marie-Tooth disease type 2A are linked to mutations in the OPA1 and MFN2 genes encoding components of the inner and outer membrane fusion machinery, respectively.[1]

In *S. cerevisiae*, mitochondrial functions are dispensable and viable mutants can be cultivated under fermentative conditions. For this reason, yeast represents an easy and powerful model for the study of the dynamics of mitochondria. Moreover, yeast is a useful model organism also to study apoptosis and aging, and mitochondrial dynamics were shown to play a role in both processes.[2]

The use of yeast mutants allowed the identification of *FZO1*, *MGM1* and *UGO1* as the main genes controlling mitochondrial fusion.

Yeast *FZO1* (mitofusin) encodes an evolutionary conserved GTPases associated to a complex localized in the outer mitochondrial membrane.

MGM1 encodes a dynamin-related GTPase corresponding to mammalian OPA1.

UGO1 encodes a protein, up to now described only in fungi, supposed to be the connector between the products of the *FZO1* and *MGM1* genes.

Mutants in these genes share a similar phenotype consisting in the presence of highly fragmented mitochondria resulting from the impairment of fusion events counteracting the fission machinery.[3,4]

It has been reported that aging yeast cells show a reduction of the mitochondrial tubular network and that the promotion of mitochondrial fusion, caused by the deletion of *DNM1* or by the incubation of cells with specific drugs, led to an increased lifespan.[5-8]

Since the absence of OPA1 is lethal in mammalian cells, an interesting contribution to this field comes from a recent paper by Oisenwacz and colleagues, published in Cell Cycle, who studied in yeast cells the effect of the absence of Mgm1 (OPA1) during aging. The authors, after separation of young and aged cells by elutriation, demonstrated that in *MGM1* mutants, that show unopposed mitochondrial fission, there is no difference in the distribution of mitochondrial morphotypes between young and old cells, as more than 95% of them contains fragmented mitochondria. On the contrary, wild-type young cells essentially show mitochondrial tubular network with reduced fragmentation, a phenomenon that increases significantly in stationary phase aging cells.

Linked to the aberrant mitochondrial morphology, the absence of Mgm1 negatively influences both chronological and replicative lifespan in yeast. Moreover, *mgm1* mutant cells are more susceptible to the induction of apoptosis by hydrogen peroxide.[9]

These results, obtained with yeast, clearly confirm the connection of mitochondrial dynamics to longevity and apoptosis, opening the possibility to attenuate age-related degenerative processes by selective down-regulation of mitochondrial fission in mammalian tissue and organs.

©2011 Landes Bioscience. Do not distribute

References

1. Cho DH, et al. Cell Mol Life Sci 2010; 67:3435-47; PMID:20577776; http://dx.doi.org/10.1007/s00018-010-0435-2.
2. Carmona-Gutierrez D, et al. Cell Death Differ 2010; 17:767-73.
3. Westermann B. Nat Rev Mol Cell Biol 2010; 11:872-84; PMID:21102612; http://dx.doi.org/10.1038/nrm3013.
4. Westermann, B. Semin Cell Dev Biol 2010; 21:542-9.
5. Cassidy-Stone A, et al. Dev Cell 2008; 14:193-204; PMID:18267088; http://dx.doi.org/10.1016/j.devcel.2007.11.019.
6. Palermo V, et al. Aging Cell 2010; 9:570-9; PMID:20550520; http://dx.doi.org/10.1111/j.1474-9726.2010.00587.x.
7. Palermo V, et al. Folia Microbiol (Praha) 2007; 52:479-83; PMID:18298044; http://dx.doi.org/10.1007/BF02932107.
8. Scheckhuber CQ, et al. Nat Cell Biol 2007; 9:99-105; PMID:17173038; http://dx.doi.org/10.1038/ncb1524.
9. Scheckhuber CQ, et al. Cell Cycle 2011; 10: 3105-10.

Notch signaling mediates p63-induced quiescence: A new facet of p63/Notch crosstalk

Comment on: Kent S, et al. Cell Cycle 2011; 10:3111–8

Nicole Forster and Leif W. Ellisen*; Massachusetts General Hospital Cancer Center and Harvard Medical School; Boston, MA USA;
*Email: ellisen@helix.mgh.harvard.edu; http://dx.doi.org/10.4161/cc.10.21.18182

Cellular quiescence, a specialized form of growth arrest, is a carefully controlled developmental program that is essential for the maintenance of normal stem cell regenerative potential. Accumulating evidence suggests that the quiescence program may be co-opted by tumor cells, increasing their long-term survival and decreasing their sensitivity to a variety of anticancer therapies. In the mammary gland, pathways involved in stem cell self-renewal include those controlled by p63, a p53-related transcription factor, and Notch, a family of proteins with diverse roles in development and cancer. In the September 15th issue of *Cell Cycle*, Kent et al.[1] provide new mechanistic insights into the maintenance of mammary cellular quiescence. They demonstrate that expression of ΔNp63α, the predominant isoform of p63 expressed in mammary epithelial cells, promotes cellular quiescence, and they provide direct evidence that activation of Notch3, one of four Notch family members expressed in mammals, is a principle mediator of p63-induced quiescence (**Fig. 1A**). These findings add a new dimension to the already substantial and complex interplay between p63 and Notch signaling in the epithelium.

An essential role for p63 in early mammary gland development is evidenced by the phenotype of constitutive *p63*-null mice, which show a complete lack of stratified squamous epithelia and their derivatives, including mammary glands, and die shortly after birth.[2,3] This dramatic phenotype has been attributed in part to a fundamental role for p63 in maintaining proliferative potential of stem cells in the epithelium,[4] which is consistent with a contribution by p63 to stem cell quiescence. In the adult mammary gland, p63 expression is restricted to the basal rather than luminal epithelial layer, and p63 expression has been shown to contribute to the induction and/or maintenance of basal cell fate.[5] Remarkably similar dual roles for Notch signaling in stem cell preservation and cell fate determination in the mammary gland have been described. Notably, however, whereas both p63 and Notch family members, including Notch3, have been linked to self-renewal of stem-like cells, Notch signaling is thought to drive commitment toward the luminal rather than basal cell fate. Indeed, in vivo models of normal mammary gland development have demonstrated the ability of p63 expression to counter Notch signaling and promote basal cell fate, while Notch activation drives luminal differentiation associated with p63 downregulation (**Fig. 1B**).[5,6] Collectively, the manuscript by Kent et al.[1] and data cited above support a model involving complex, reciprocal regulation of p63 and Notch in the mammary gland.

How do we reconcile experiments demonstrating positive regulation vs. those showing negative reciprocal regulation of p63 and Notch expression and function? One possible explanation is that Notch-stimulating and -repressing activities of p63 may in fact be occurring in different cells. For example, in addition to regulating expression of Notch itself, p63 is also known to regulate expression of Notch ligands, which could control Notch signaling in a non-cell-autonomous manner. Furthermore, selective regulation of different Notch family members by p63 may be involved, as Notch1 and Notch2 are expressed at higher levels in luminal rather than basal cells, whereas Notch3 and Notch4 are comparably or possibly more highly expressed in basal epithelial cells.[6] Analysis of distinct mammary cell populations derived from in vivo studies should help clarify the details of Notch and p63 cross regulation.

Further work will also be required in order to understand the fascinating association of p63 and Notch signaling pathways with quiescence and preservation of early stem/progenitor cells on the one hand, and with proliferation and differentiation of later committed progenitors on the other (**Fig. 1**).[4-6] This dichotomy could again be explained by differential expression and functions for different Notch family members in distinct developmental stages[6] or, in the case of p63, by differential isoform expression. Another possibility involves expression levels, as suggested by a recent study that used an in vitro three-dimensional model to show that high

©2011 Landes Bioscience
Do not distribute

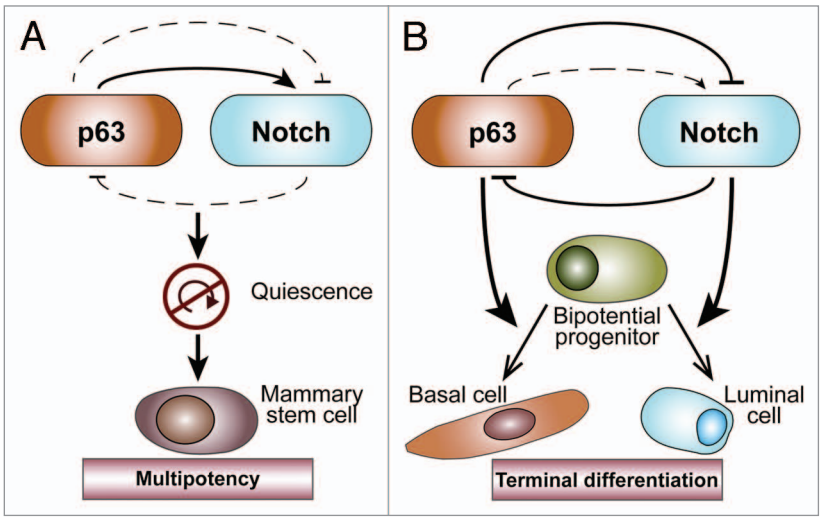


Figure 1. Stage-specific regulation and function of p63 and Notch signaling in the mammary gland. (A) Activation of Notch expression by p63 inhibits cell cycle progression, thereby promoting quiescence which is associated with self-renewal of mammary stem cells. (B) In contrast, reciprocal inhibition of p63 and Notch signaling controls cell fate determination of bipotential progenitor cells along basal (p63-associated) or luminal (Notch-associated) epithelial lineages.

Notch activity inhibits proliferation, whereas low Notch activity induces a hyperproliferative response.[7] Both cellular context and other collaborating signal transduction pathways are also likely to contribute to this stage-specific phenotypic output of Notch and p63 activation. In this regard, it is intriguing that genetic evidence points to context-specific contributions of p63 and Notch to both tumorigenesis and tumor suppression.[8,9] Unraveling the emerging web of crosstalk between p63 and Notch will be essential if we are to exploit the potential therapeutic benefit of targeting these two pathways in human cancer.

References

1. Kent S, et al. Cell Cycle 2011; 10:3111-8; PMID:21912215; http://dx.doi.org/10.4161/cc.10.18.17300
2. Mills AA, et al. Nature 1999; 398:708-13; PMID:10227293; http://dx.doi.org/10.1038/19531.
3. Yang A, et al. Nature 1999; 398:714-8; PMID:10227294; http://dx.doi.org/10.1038/19539.
4. Senoo M, et al. Cell 2007; 129:523-36; PMID:17482546; http://dx.doi.org/10.1016/j.cell.2007.02.045.
5. Yalcin-Ozuysal O, et al. Cell Death Differ 2010; 17:1600-12; PMID:20379195; http://dx.doi.org/10.1038/cdd.2010.37.
6. Bouras T, et al. Cell Stem Cell 2008; 3:429-41; PMID:18940734; http://dx.doi.org/10.1016/j.stem.2008.08.001.
7. Mazzone M, et al. Proc Natl Acad Sci USA 2010; 107:5012-7; PMID:20194747; http://dx.doi.org/10.1073/pnas.1000896107.
8. Flores ER. Cell Cycle 2007; 6:300-4; PMID:17264676; http://dx.doi.org/10.4161/cc.6.3.3793.
9. Leong KG, et al. Blood 2006; 107:2223-33; PMID:16291593; http://dx.doi.org/10.1182/blood-2005-08-3329.

Conversion from senescent cells to pluripotent cells by modulating expression of *Alu* retroelements

Comment on: Wang J, et al. Cell Cycle 2011; 10:3016–30

Osamu Iwasaki, Christopher J. Corcoran and Ken-ichi Noma*; The Wistar Institute; Philadelphia, PA USA; *Email: noma@wistar.org;

http://dx.doi.org/10.4161/cc.10.21.18184

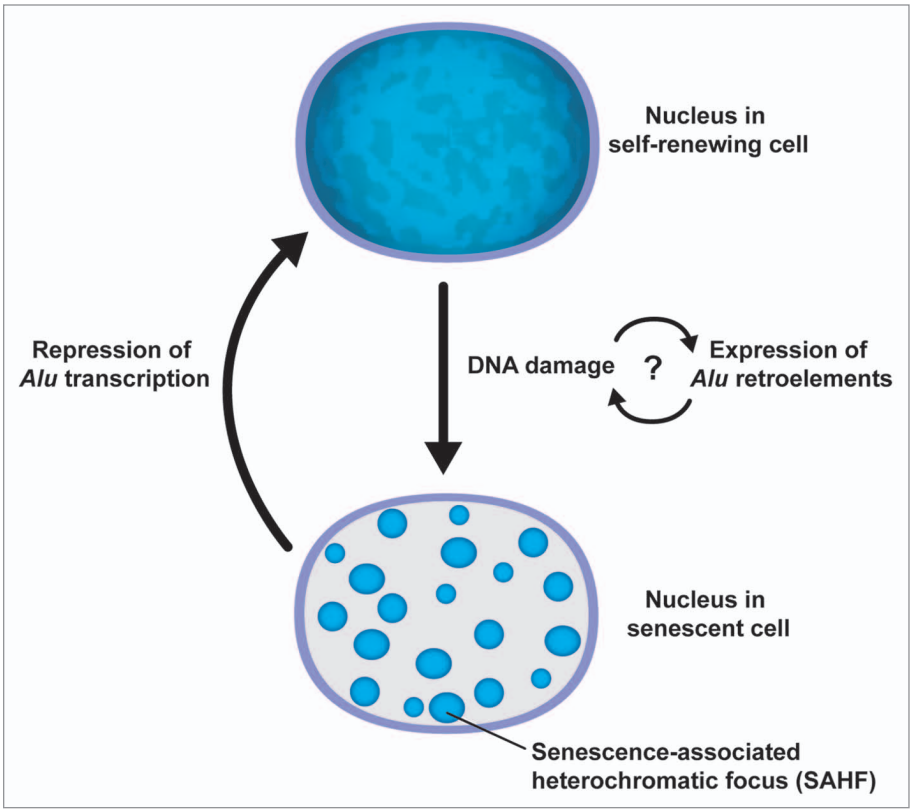
Epigenetic chromatin regulations in aging have been intensely studied.[1] During aging, overall DNA methylation tends to decrease, although some loci are specifically methylated. Histone modifications across the genome are also known to be modulated during aging. For instance, the globular accumulation of heterochromatin associated with methylation of histone H3 at lysine 9 and heterochromatin protein HP1, referred to as senescence-associated heterochromatic foci (SAHFs), was observed in senescent human fibroblasts.[2] However, the exact roles of epigenetic chromatin regulations in cellular senescence remain elusive. As an additional layer of complexity, DNA damage is known to be accumulated during aging.

It was previously shown that DNA damage induced by chemical treatments can derepress SINE (short interspersed element) retrotransposons, such as *Alu*, in human cells.[3] Wang et al. set out to test the possibility that DNA damage during aging causes derepression of the *Alu* retroelements.[4] The authors employed the ex vivo aging system with human adult adipose-derived mesenchymal stem cells (hADSCs), and found that *Alu* expression is dramatically increased in senescent cells with formation of γH2AX (phosphorylated histone variant H2AX) nuclear foci. The γH2AX is recruited to DNA damage sites and mediates the DNA repair process. Interestingly, more than 60% of γH2AX peaks were assigned to *Alu* (SINE), *L1* (LINE) and LTR retrotransposons. Moreover, 53BP1, the mediator of the DNA damage checkpoint, is colocalized with a particular centromere in senescent cells. It is possible that retroelements associated with DNA damage might be clustered at the centromere. To support this speculation, cohesin and condensin, which are known to play roles in higher-order chromatin organization,[5] bind to the *Alu* retroelements. It was previously shown that the SNF2h-chromatin remodeling complex loads cohesin onto the *Alu* retroelements.[6] In fission yeast, RNA polymerase III (Pol III)-transcribed genes, such as *tRNA* and *5S rRNA* genes, distributed across the genome, physically associate with centromeres.[7] Since *Alu* retroelements are also transcribed by Pol III, it is possible that a similar genome-organizing mechanism governs both the *Alu*-mediated genome organization in human and the centromeric localization of Pol III genes in fission yeast.

Is *Alu* transcriptional activation, in fact, directly related to cellular senescence? Remarkably, the stable knockdown of *Alu* transcription can convert senescent cells into proliferative cells (**Fig. 1**).[4] This conversion is concomitant with upregulation of pluripotency factors Nanog and Oct4, implying that *Alu* transcription might be a major factor in cellular senescence, and that *Alu* repression contributes to pluripotency.

Alu repression can reverse the senescent phenotype of human cells. In the model, the authors proposed that upregulation of *Alu* transcription might facilitate DNA damage at the *Alu* retroelements, which, in turn, is detected by the DNA damage checkpoint and causes senescence. This study raises two interesting questions. First, how is *Alu* transcription activated in senescent cells? It might involve hSNF2 loading to the *Alu* retroelements. Other possible mechanisms might be involved with dysregulation of post-transcriptional silencing for *Alu* retroelements. It was shown that Dicer1 expression is decreased in aging-related macular degeneration.[8] Dicer1 is essential for post-transcriptional gene silencing through the processing of transcripts into small RNAs. Thus, Dicer1 deficiency results in accumulation of unprocessed *Alu* transcripts, leading to *Alu* RNA toxicity and human pathology.[8] Second, can a chemical inhibitor that represses *Alu* transcription decelerate cellular aging? It was shown that rapamycin treatment that can inhibit expression of Pol III genes, including *Alu* retroelements, decelerates senescence in human cells.[9] In conclusion, further study beyond these initial experiments may lead to the application of pharmacological compounds in order to decelerate senescence and cell aging in the human body.

©2011 Landes Bioscience
Do not distribute



©2011 Landes Bioscience.

Figure 1. Role of *Alu* retroelements in cellular senescence. The schematic model shows the DAPI staining patterns of the nuclei in self-renewing and senescent human cells. DNA damage and *Alu* transcripts are accumulated in senescent cells. Repression of *Alu* retroelements by shRNA knockdown is sufficient to convert senescent cells to self-renewing cells.

References

1. Calvanese V, et al. Ageing Res Rev 2009; 8:268-76; PMID:19716530; http://dx.doi.org/10.1016/j.arr.2009.03.004.
2. Narita M, et al. Cell 2003; 113:703-16; PMID:12809602; http://dx.doi.org/10.1016/S0092-8674(03)00401-X.
3. Rudin CM, et al. Genes Chromosomes Cancer 2001; 30:64-71; PMID:11107177; http://dx.doi.org/10.1002/1098-2264(2000)9999:9999<::AID-GCC1066>3.0.CO;2-F.
4. Wang J, et al. Cell Cycle 2011; 10:3016-30; PMID:21862875; http://dx.doi.org/10.4161/cc.10.17.17543.
5. Losada A, et al. Genes Dev 2005; 19:1269-87; PMID:15937217; http://dx.doi.org/10.1101/gad.1320505.
6. Hakimi MA, et al. Nature 2002; 418:994-8; PMID:12198550; http://dx.doi.org/10.1038/nature01024.
7. Iwasaki O, et al. Mol Biol Cell 2010; 21:254-65; PMID:19910488; http://dx.doi.org/10.1091/mbc.E09-09-0790.
8. Kaneko H, et al. Nature 2011; 471:325-30; PMID:21297615; http://dx.doi.org/10.1038/nature09830.
9. Demidenko ZN, et al. Cell Cycle 2009; 8:1888-95; PMID:19471117; http://dx.doi.org/10.4161/cc.8.12.8606.

RNA processing in a tiny transcriptome

Comment on: Lopez Sanchez MI, et al. Cell Cycle 2011; 10:2904–16

Christiane Kupsch and Christian Schmitz-Linneweber*; Humboldt University of Berlin; Berlin, Germany;
*Email: smitzlic@rz.hu-berlin.de; http://dx.doi.org/10.4161/cc.10.21.18185

Mitochondria are the descendants of free-living α-proteobacteria. Their genomes have shrunk to a tiny set of genes that, in humans, encode only 13 mRNAs, the 12S and 16S rRNAs and 22 tRNAs. These genes are essential, but they are far from sufficient to enable respiration and mitochondrial gene expression. Instead, mitochondria import cytosolic help in the form of hundreds of additional nuclear-encoded factors. While the number of nuclear factors known to be involved in mitochondrial gene expression has increased sharply in the past few years, we know relatively little about the mechanistic properties of these factors. A case in point is the topic of mitochondrial RNA processing in humans: we know that human mitochondrial genomes are transcribed from three promoters that give rise to two long polycistronic transcripts covering the entire genome and a third shorter transcript encompassing the two rRNAs and one tRNA. These transcripts are broken down to yield mature mRNAs, tRNAs and rRNAs.

The prevailing model for how this processing occurs is now 30 y old and, in a nutshell, says that the excision of tRNAs splits polycistronic transcripts into monocistronic units. This model, which is known as the tRNA punctuation model of human mitochondrial gene expression,[1] implies that tRNA maturation is crucial for mitochondrial RNA processing. Although studies showed that tRNA end processing is performed in most organisms by RNase P (for the 5′ end) and RNase Z (for the 3′ end), it was long unclear which proteins played these roles in human mitochondria. Recently, however, a biochemical characterization of mitochondrial RNase P activity uncovered a non-canonical three-subunit complex consisting of the proteins MRPP1, -2 and -3.[2] Furthermore, results from other studies suggested that ELAC2 could be the RNase Z homolog in humans.[3] How these proteins affect overall RNA processing in human mitochondria in vivo remained unclear.

In the September 1st issue of *Cell Cycle*, Lopez Sanchez et al.[4] reported having investigated the function of the aforementioned proteins by combining classical molecular biology techniques with a next generation sequencing (NGS) analysis of mitochondrial transcripts. For the first time, the NGS approach allowed the authors to both comprehensively and quantitatively examine changes in human mitochondrial transcript processing. They report that knockdown of MRPP1 and MRPP3 decreases the abundance of most tRNAs and, furthermore, decreases the proportion of tRNAs with mature 5' ends without affecting the distribution of mature 3′ ends. In contrast, knockdown of ELAC2 decreases the proportion of rRNAs with mature 3′ ends but does not affect the 5′ ends. The authors confirm these processing defects using classical qRT-PCR analyses that demonstrate a striking overaccumulation of unprocessed precursor RNAs over mature transcripts in the knockdown cells. These results provide striking experimental support for the hypotheses that MRPP1, MRPP3 and ELAC2 are involved in mitochondrial tRNA maturation and are thus crucial to overall mitochondrial RNA processing. In addition to their detailed characterization of known factors, the authors also uncovered a novel general player in tRNA maturation, PTCD1. Although PTCD1 was previously described as associating with incompletely processed tRNAs and was believed to be involved in regulating the abundance of the leucine tRNA,[5] the NGS results presented by Lopez Sanchez et al. suggest that PTCD1 actually targets the processing of many tRNAs. Furthermore, the co-purification of PTCD1 and ELAC2 suggest that these proteins may form a general machine for the processing of tRNA 3′ ends.

Given that mitochondria and, in particular, the genetic information encoded within mitochondria are key players in aging, cancer, diabetes and heart disease,[6,7] the Sanchez study can be seen as an exciting new jumping-off point for a detailed analysis of the human mitochondrial RNA processing machinery. While the overexpression experiments presented by Lopez Sanchez et al. suggest that the investigated factors are not rate limiting for RNA processing in the tested cell lines and under the utilized conditions, future analyses should thoroughly test these findings in vivo. In addition, future work can address the pressing unanswered questions, which include, how do the identified proteins mechanistically foster RNA processing? Are they true regulators of gene expression? Surely, this is an exciting time to be examining the tiny transcriptome of the human mitochondria!

©2011 Landes Bioscience
Do not distribute

References

1. Ojala D, et al. Nature 1981; 290:470-4; PMID:7219536; http://dx.doi.org/10.1038/290470a0.
2. Holzmann J, et al. Cell 2008; 135:462-74; PMID:18984158; http://dx.doi.org/10.1016/j.cell.2008.09.013.
3. Takaku H, et al. Nucleic Acids Res 2003; 31:2272-8; PMID:12711671; http://dx.doi.org/10.1093/nar/gkg337.
4. Lopez Sanchez MI, et al. Cell Cycle 2011; 10:2904-16; PMID:21857155; http://dx.doi.org/10.4161/cc.10.17.17060.
5. Rackham O, et al. Nucleic Acids Res 2009; 37:5859-67; PMID:19651879; http://dx.doi.org/10.1093/nar/gkp627.
6. Wallace DC. Annu Rev Genet 2005; 39:359-407; PMID:16285865; http://dx.doi.org/10.1146/annurev.genet.39.110304.095751.
7. Shutt TE, et al. Environ Mol Mutagen 2010; 51:360-79; PMID:20544879.